REMARKS

Claim 8 is pending in this application. Claim 8 has been rejected. Reconsideration is respectfully requested in light of the following remarks.

I. Rejection Under 35 U.S.C. §103

Claim 8 has been rejected under 35 U.S.C. 103(a) as being unpatentable over Bard *et al.* (1977) in view of Yoshida *et al.* (1996), as evidenced by Adamsom (1996). The Examiner suggests that Bard *et al.* teaches that at higher than physiological concentrations (2, 5, 10 and 20 micromolar) retinoids destabilize membranes causing the release of lysosomal enzymes and the effect can be followed by metachromatic staining and by measuring the appearance of proteoglycan fragments in the medium of organ cultures of rabbit ear cartilage. The Examiner acknowledges that this reference fails to teach a method comprising contacting a cell expressing PML/RARα and detecting whether said agent destabilizes lysosomes of the cell as determined by vital staining of lysosomes or release of lysosomal proteins into the cytosol, as well as increasing lysosomal-dependent PML-RARα degradation. However, the Examiner suggests it would have been obvious for one of ordinary skill in the art to combine the teaching of Bard et al. with the teaching of Yoshida et al. (1996) where Yoshida et al. teaches contacting an APL cell that expresses PML/RARα with an anti-cancer agent, specifically ATRA, at concentrations of 1, 0.1 and 0.01 micromolar, and detecting whether ATRA increases PML/RARα protein degradation to arrive at the method of the present invention. Thus, the Examiner suggests that one of skill would understand that a dose of 1, 2 or 5 micromolar

as taught by Bard et al. (1977) would constitute an effective amount of ATRA as it falls within the clinically useful concentrations of Adamson (1996). The Examiner suggests that one of skill would have been aware that as lysosomes destabilize they release lysosomal enzymes into the cytosol which would actively degrade any proteins they encounter, including PML/RARα. Thus, the Examiner suggests that the limitation that the protein be "lysosomal-dependent" is inherently met as destabilization of the lysosomes releases enzymes and would not otherwise occur, making the resulting degradation necessarily lysosomal dependent. The Examiner also suggests that arguments of the Applicants are not persuasive since both Bard et al. (1977) and Yoshida et al. (1996) teach effective amounts of ATRA that fit within the broad definition of effective amount within the specification as filed.

Applicants respectfully traverse this rejection.

As discussed in the previous Reply dated May 12, 2011, Bard *et al.* (1977), as acknowledged by the Examiner, discloses investigations into the toxicity of retinoids in a rabbit model and understanding the mechanism of retinoid-induced toxicity in tissues such as cartilage. The retinoids were administered at doses of from 2 to 20 μM, doses or concentrations that one of skill in the art would know produce toxicity, not doses that are clinically useful since induction of toxicity is an undesired clinical result. In fact, this specific issue of undesirable toxicity and its role in development of clinically useful anti-cancer drugs is discussed by Bard *et al.* at page 118 where it is stated:

> *The results support the hypothesis that retinoic acid may act as a detergent in destabilizing lysosomal*

> *membranes (Dingle and Fell, 1963), since all the compounds with carboxylic acid groups are active, and the replacement of the acid moiety by less hydrophilic groups would diminish their efficiency as surfactants. The results also imply that in cartilage, at least, ethers, esters and amides are not converted extensively to retinoic acid, The possibility that these conversions may take place in other tissues, and consequently affect the pharmacology of these compounds, cannot be excluded ay present.*
>
> *The anti-carcinogenic activity appears to be unrelated to the interaction with membranes, since the most effective anti-carcinogens are not necessarily the most polar (Lasnitzki, 1976), and detergents of different structures do not oppose tumour development (Lasnitzki and Goodman, 1974). The identification of compounds which combine potent anti-carcinogenic action with low activity in destabilizing lysosomes offers considerable hope for the eventual development of an effective anti-tumour agent of low general toxicity.[emphasis added]*

Clearly, Bard *et al.* teach that retinoic acid itself possesses a toxicity profile that involves lysosomal destabilization and that such lysosomal destabilization is not an attribute that would be sought by one of skill in the art when developing retinoic acid analogs as anti-cancer drugs, or in screening drugs for ability to inhibit cancer cell growth, such as is claimed in the instant invention.

"A reference may be said to teach away when a person of ordinary skill, upon reading the reference, would be discouraged from following the path set out in the reference, or would be led in a direction divergent from the path that was taken by the applicant." *In re Gurley*, 27 F.3d 551, 553 (Fed. Cir. 1994). Bard *et al.* clearly teach away

from the method of the present invention which relies on the ability of an agent to destabilize lysosomes within a cell as a desired drug effect. Thus, the reference of Bard et al. not only fails to teach or suggest all limitations of the instant claims but also teaches away from the instant method and as such would not provide one of skill with either a motivation to combine this teaching with other prior art or to expect success when combining the teaching with other art to arrive at a method of identifying agents that could be used clinically to treat cancer.

Also as previously discussed, Yoshida *et al.* (1996), when combined with Bard *et al.* (1977) fails to teach the limitations of the claim as previously amended. In previous replies filed during prosecution of the case, Applicants respectfully pointed to the paragraph spanning pages 2946-2947 of Yoshida *et al.* which states:

> Accordingly, we supposed that PML-RARA bound with ATRA might become unstable and undergo accelerated degradation. The degradation of most cellular proteins is catalyzed by the nonlysosomal ubiquitin-proteasome pathway, which is dependent on ATP and closely involved in the proteolysis of aberrantly generated products (13). To investigate whether proteasomes are involved in the decrease of PML-RARA by ATRA, we examined the effect of the *Streptomyces* metabolite lactacystin (14), a highly specific inhibitor of the proteasome (15). The decrease of PML-RARA induced by ATRA was dose-dependently inhibited by lactacystin (Fig. 4). Lactacystin at 10 μM almost completely inhibited the decrease of PML-RARA induced by 1 μM ATRA. These data suggest that ATRA accelerates the degradation of PML-RARA in the proteasome pathway.

The cited passage specifically teaches that "ATRA accelerates the degradation of PML-RARα in the proteasome

pathway." This is in direct contrast to the instant invention, which is directed to the identification of agents that induce the lysosome-dependent degradative pathway (page 5, lines 1-26 of the instant specification). Indeed, as described at page 5, lines 10-11, proteasome and caspase inhibitors do not block PML/RARα degradation in accordance with the instant assay. In this respect, the instant assay requires the identification of agents that both destabilize lysosomes and increase PML/RARα protein degradation. It is only with the specification in hand that one of skill would be motivated to consider developing a method as claimed which involves contacting a cell that expresses PML/RARα with an effective amount of an agent, wherein an effective amount of said agent is a concentration that is clinically useful, and detecting whether said agent first destabilizes lysosomes of the cell, and second increases lysosomal-dependent PML/RARα protein degradation.

Thus, also when considering Yoshida *et al.*, "A reference may be said to teach away when a person of ordinary skill, upon reading the reference, would be discouraged from following the path set out in the reference, or would be led in a direction divergent from the path that was taken by the applicant." *In re Gurley*, 27 F.3d 551, 553 (Fed. Cir. 1994). Again, in the instant case, the whole of the teachings of Yoshida *et al.* show that PML/RARα is degraded via the proteasome pathway, *i.e.* a non-lysosomal pathway. Thus, Yoshida *et al.* unquestionably teach away from doing what Applicants have done; *i.e.*, determining whether an agent destabilizes lysosomes and increases lysomal-dependent PML/RARα protein degradation. Accordingly, there would be no motivation to combine this reference with the

teachings of Bard *et al.* for determining whether an agent destabilizes lysosomes, in particular considering the teachings of Bard *et al.* with regard to toxicity associated with lysosomal degradation and the property making an agent not useful clinically.

The Examiner further points to the teachings of Adamson (1996) suggesting that this reference discloses that the doses of ATRA taught by both Bard et al. (1977) and Yoshida et al. (1996) fall within the defined range of "clinically effective doses" that are recited in claim 8 as now presented. However, careful review of this reference shows that this is not the case. As discussed at the section on page 307 referred to by the Examiner, peak plasma concentrations of ATRA in patients vary widely. In fact, nowhere does this paper teach that any specific range of plasma concentration is clinically effective, instead teaching at page 307 that patients treated with ATRA "invariably relapse" after the start of treatment and that plasma concentrations "diminish rapidly" with daily dosing. Nowhere does this paper tech that the concentrations of Bard et al. (1977), which are in the range of 1-5 micromolar, would be the plasma level needed to be achieved to be clinically useful. In fact, teachings of Adamson (1996) in another part of the paper show very different measures for clinically useful levels of ATRA in patients which are based on AUC levels in patients, or measures of total systemic exposure over time, NOT a single plasma level (see page 311, first column). At page 311, first column, it is taught:

> "Based on preclinical studies [47], we investigated intermittent schedules of ATRA administration, including a dosing schedule of 7 days on/7 days off [44]. In this study, the plasma AUC declined

> significantly during the first week of drug administration, from a mean (± SD) AUC of 145 ± 26 M•min on days 1 to 18 ± 4 μM•min on day 7. Plasma ATRA concentrations at the start of weeks 3 and 11 of this every other-week schedule, however, were equivalent to those achieved on day 1 of treatment. Mean AUCs were 177 ± 39 and 128 ± 30 μM•min on day 1 of weeks 3 and 11, respectively (Fig. 7). This rapid upregulation of ATRA clearance followed by downregulation during the phase of the intermittent schedule when the patient is off therapy has been observed in other studies [71, 75, 76]. Thus, intermittent schedules of ATRA administration appear to circumvent the low plasma drug exposure that results from the sustained upregulation of catabolism on chronic daily dosing schedules."

The discussion in the paper of Adamson (1996), thus, is not defining a specific clinically useful range for drug levels in blood but instead is addressing the problem encountered with ATRA that is related to the rapid development of drug resistance, which makes identifying clinically useful drug levels in blood almost impossible based on the treatment regimens that were in use in 1996. In fact, this is confirmed by the conclusions to the paper as follows:

> "An understanding of the clinical pharmacology of ATRA has been an area of intensive research, as it initially appeared to be linked to treatment failure. Our current knowledge suggests that ATRA resistance is not simply an inability to maintain therapeutic plasma concentrations of drug, but rather may be linked to the intracellular regulation of drug. The intricate nature of the homeostatic mechanisms that maintain tight control over retinoids, combined with the multiplicity of retinoid receptors and signaling pathways, leave open the possibility of a yet-to-be-defined mechanism of resistance that is independent of the clinical pharmacology of ATRA."

As a result, one of skill in the art would not see the teachings of Adamson (1996) as evidence that any teaching of

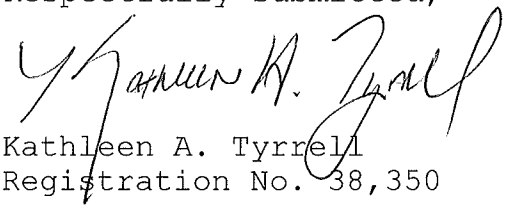
Bard et al. (1977) or Yoshida et al. (1996) is a teaching of a clinically useful level of ATRA in blood.

Based on the deficiencies in teaching of the combined references concerning the use of doses that are clinically useful, and the fact that the references of Bard et al. (1977) and Yoshida et al. 1996) each teach away from the instant method, Applicants respectfully assert that the basis of this rejection is not supported by the whole of the teachings of the cited references. Thus, the combined teachings of Bard *et al.* (1077) and Yoshida *et al.* (1996), as evidenced by Adamson (1996), cannot be held to make the present invention of claim 8 obvious under 35 U.S.C. 103(a). It is therefore respectfully requested that this rejection be reconsidered and withdrawn.

II. Conclusion

Applicants believe that the foregoing comprises a full and complete response to the Office Action of record. Accordingly, favorable reconsideration and subsequent allowance of the pending claims is earnestly solicited.

Respectfully submitted,



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